Abstract

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Title of diploma thesis: Preparation of mammalian vectors encoding selected aldo-keto reductases

Aldo-keto reductases 1B1, 1B10 and 1C1 (AKR1B1, AKR1B10, AKR1C1) are NADPH-dependent enzymes metabolizing a broad spectrum of carbonyl-bearing substrates. These enzymes from the superfamily of aldo-keto reductases mediate the reduction of endogenous compounds such as hormones and sugars as well as various xenobiotics and drugs. Beside their physiological roles, they significantly participate in the genesis of severe diseases (e.g. cancer, diabetes etc.). Inhibition of AKR1B1, AKR1B10 and AKR1C1 is accepted as promising therapeutic approach for the treatment of these diseases. The goal of the present work was to prepare mammalian vectors encoding AKR1B1, AKR1B10 and AKR1C1.

Primer design and cDNA amplification were the first steps. Primers contained the sequence recognized by restriction endonucleases present in the multicloning site of the pCI plasmid which allowed for the generation of cohesive ends and the consequent joining of insert with plasmid by ligation. Prepared vectors were transformed into *Escherichia coli* HB101 bacteria. Fruitfulness of insert cloning was verified by electrophoresis and control and back restriction in plasmids isolated from chosen colonies. Plasmids with a potential for successful encloning of insert were amplified, isolated and purified in midi format and verified by sequencing analysis.

Mammalian pCI vectors encoding AKR1B1, AKR1B10 and AKR1C1 were successfully prepared. These vectors will be utilized in the future for transfections in studies focused on the characterization of inhibitory and substrate affinity of selected enzymes.